Total Synthesis of 4-*Epi*-A83586C. Epimerisation in a Macrolactamisation Mediated by BOP and DMAP

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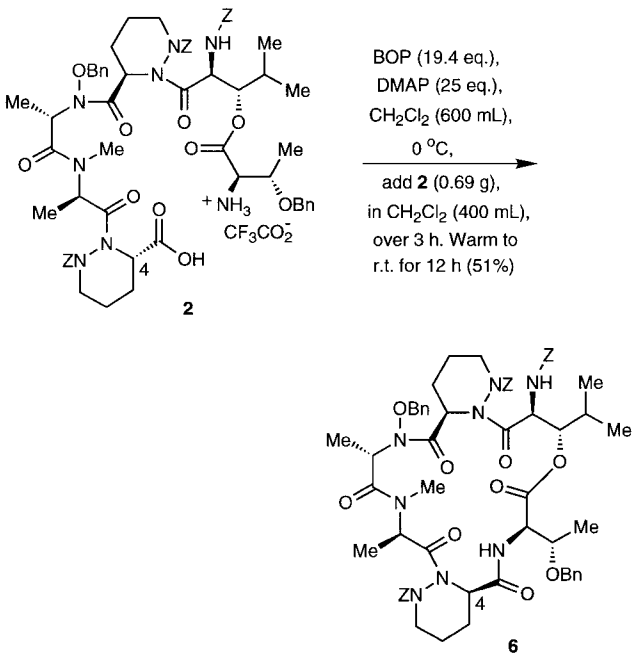
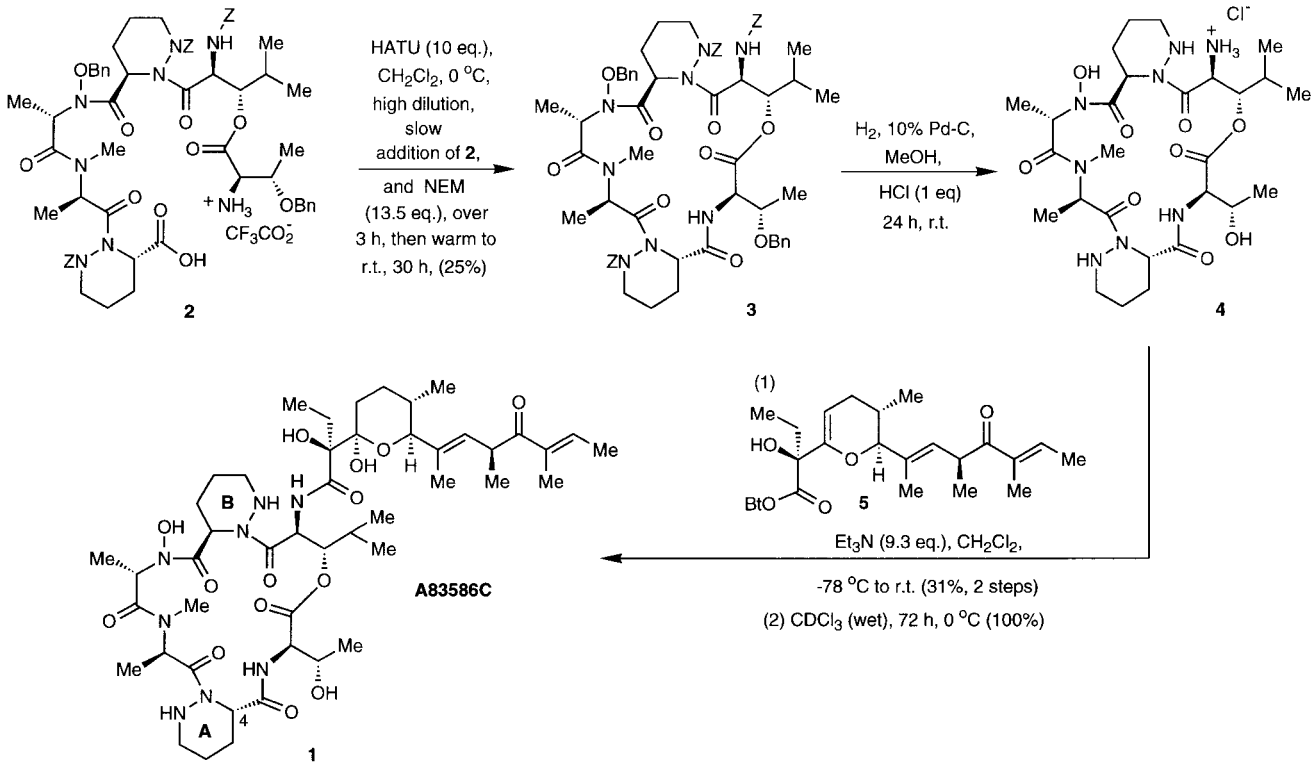
Abstract: Protected linear hexapeptide **2** undergoes a remarkably facile C(4)-epimerisation when macrolactamisation is attempted with BOP [benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate] and DMAP in CH_2Cl_2 under conditions of high-dilution; compound **6** is isolated in 51% yield from this reaction. In order to confirm its structure, **6** was independently synthesised from **18** by macrolactamisation with HATU [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate)] and NEM (*N*-ethylmorpholine); ring-closure now proceeded in 70% yield. After subsequent deprotection by catalytic hydrogenolysis, amine salt **7** was chemoselectively coupled to activated ester **5** to give 4-*epi*-A83586C (**8**) after glycal hydration.

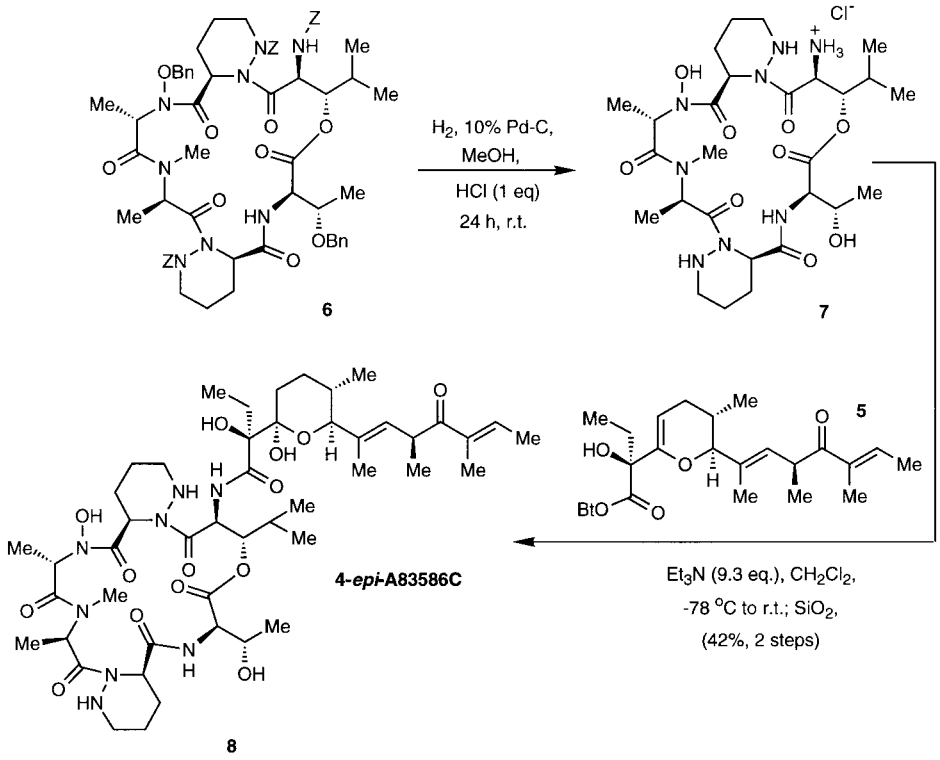
Recently we completed[1] the first asymmetric total synthesis of antitumour antibiotic A83586C (**1**) via the chemoselective coupling strategy shown in Scheme 1. A key feature of our approach was the HATU[2]/NEM mediated macrolactamisation of **2** to obtain **3** in 25% yield. In an effort to improve the yield of this cyclisation, we decided to evaluate the performance of BOP[3] and DMAP in this capacity (Scheme 2). To our surprise, rather than markedly enhancing the yield of **3**, as had been intended, this new reagent partnership actually afforded an alternative major product **6** in 51% yield. Particularly noteworthy was the small quantity of **3** now present in the reaction mixture. In essence, a



Scheme 2



Scheme 1



Scheme 3

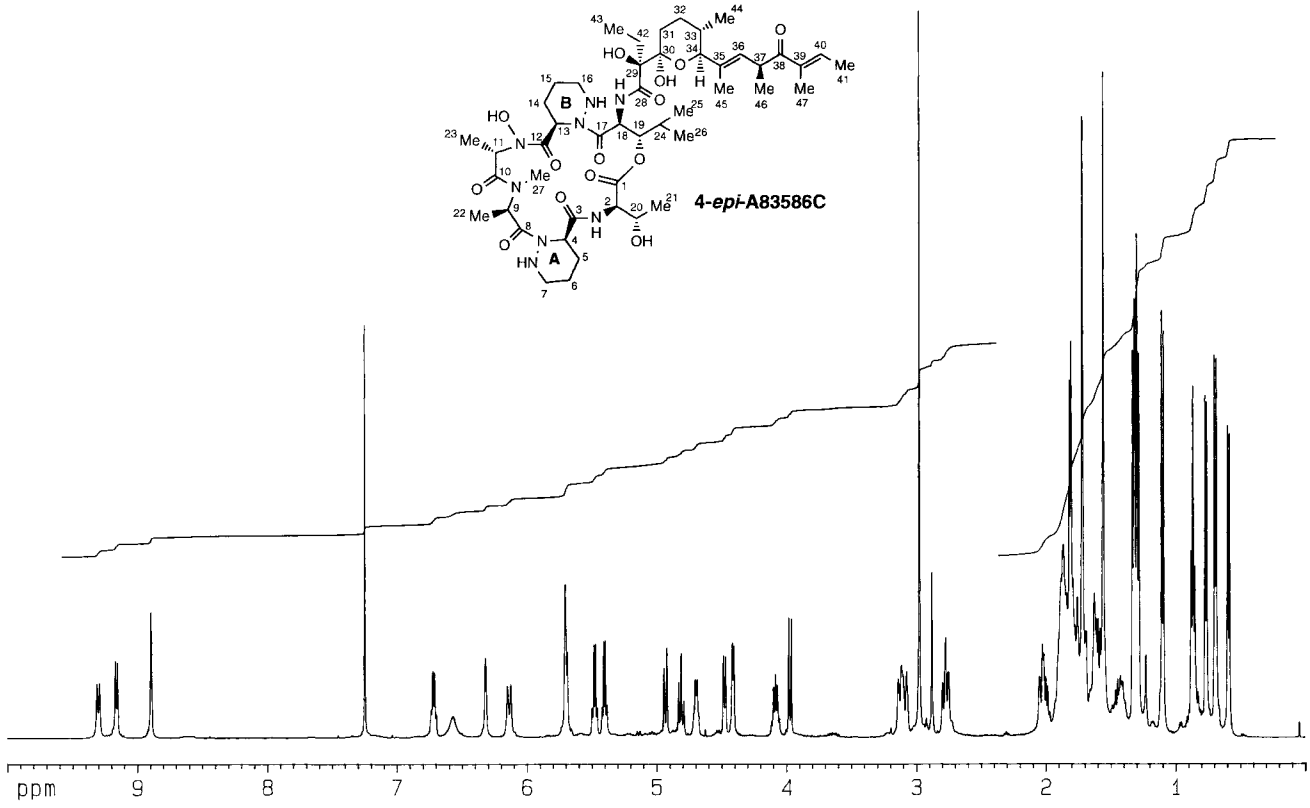
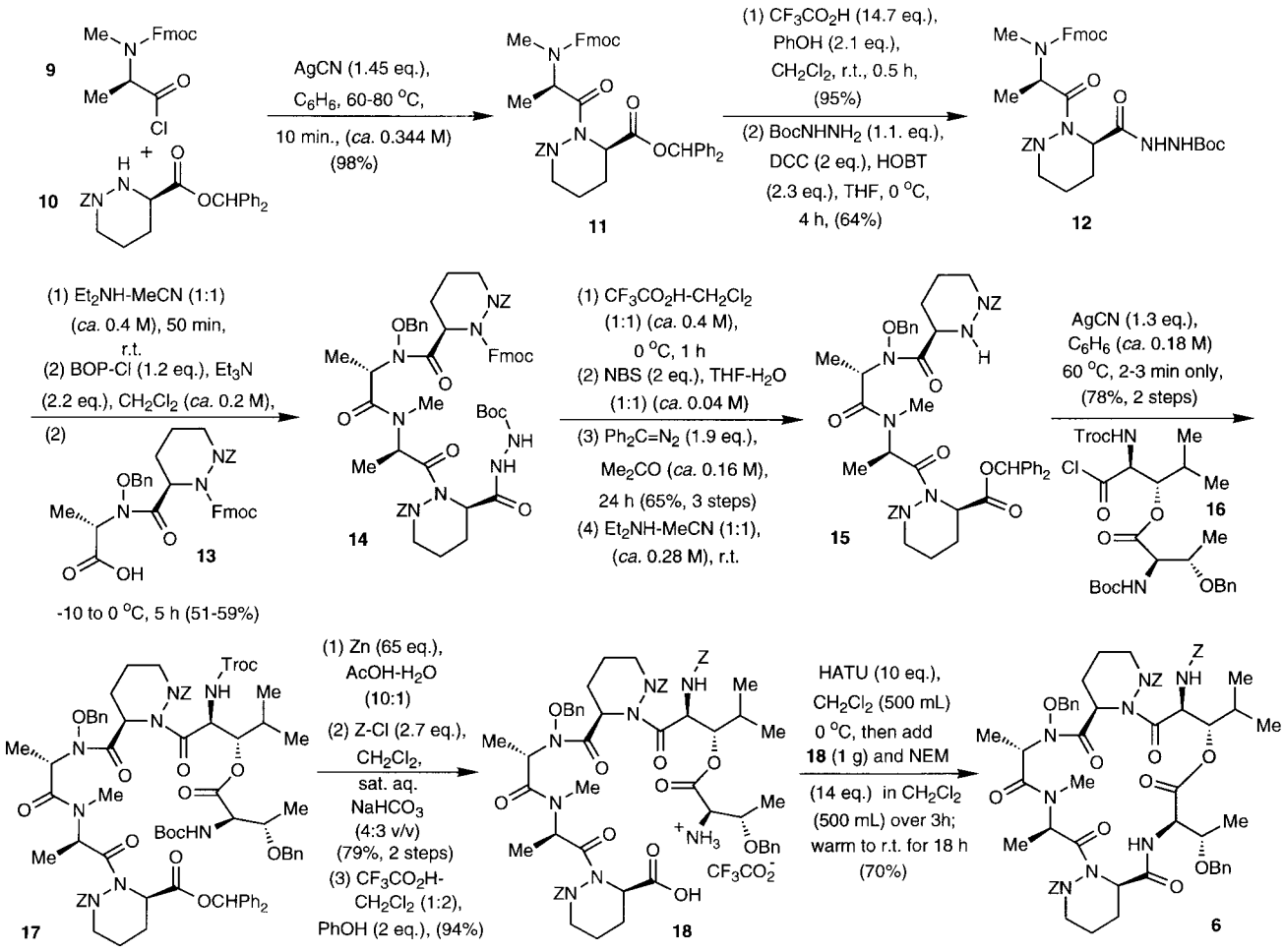


Figure 1

virtually complete epimerisation had occurred in the L-piperazic acid unit of **2** under the BOP/DMAP activation conditions used to effect cyclisation. Presumably, the enhanced basicity of DMAP, and the likely formation of acyl pyridinium intermediates, contribute to the readiness with which H(4) is abstracted and the ease of epimerisation. Whilst obviously not useful for the synthesis of A83586C, this chance observation did nevertheless lend itself to a synthesis of the novel analogue, 4-*epi*-A83586C (**8**), for biological evaluation. In this Letter



Scheme 4

we report on the first asymmetric total synthesis of **8** (Scheme 3) and show how its structure was independently confirmed by a second total synthesis. We also catalogue how 2D NMR and computer-assisted molecular modelling were used to determine a preliminary solution structure for **8**.

Compound **6** derived from the aforementioned BOP[3]/DMAP mediated cyclisation was globally deprotected by catalytic hydrogenolysis in methanolic HCl (Scheme 3). The resulting crude hydrochloride salt **7** was suspended in dry CH_2Cl_2 along with activated ester **5**,[4] and the mixture cooled to -78 °C, prior to excess Et_3N being added. Upon warming to room temperature a smooth coupling occurred to provide 4-*epi*-A83586C (**8**) in 42% yield after aqueous work up and SiO_2 flash chromatography. The 500 MHz ^1H NMR spectrum of **8** in $CDCl_3$ is shown in Fig.1.

Since high field NMR spectroscopy was unable to unambiguously prove that epimerisation had occurred at C(4) in **8**, we decided to carry out an independent total synthesis of **6** in order to confirm its structure; our route is presented in Scheme 4. Known acid chloride **9**[5] (1.17 eq.) was reacted with (3*R*)-piperazic acid derivative **10**[5,6] (1 equiv) in dry benzene at 60-80 °C, in the presence of AgCN[7] (1.45 eq.); dipeptide **11** was obtained in 98% yield. To side-step the potential problem of diketopiperazine formation occurring during the Fmoc cleavage of **11**, we temporarily replaced the diphenylmethyl ester group[8] with a BOC-hydrazide group.[9] This enabled the Fmoc group to be cleanly detached from **12**, and a coupling attempted with **13**[5] using BOP-Cl[10] and Et_3N in CH_2Cl_2. This furnished tetrapeptide **14** in 51% yield from **12**. Compound **14** was then converted into **15** in a further four steps and this condensed with known acid chloride **16**[1] using the AgCN coupling method of Durette *et al.*[7] The Fmoc[11] deprotection and amidation steps delivered hexapeptide **17** in a combined yield of 78%. The next phase of the synthesis was Troc to Z interconversion,[1] followed by acidolysis, to furnish **18**. To our surprise, the latter cyclised efficiently when added slowly with NEM in CH_2Cl_2 to HATU[2] in CH_2Cl_2 at 0 °C, under conditions of high dilution. Cyclodepsipeptide **6** was isolated in 70% yield after flash chromatography. The high yield observed in this macrolactamisation seemed especially noteworthy after one considered the low yield recorded for the cyclisation of **2** (25%). The latter reaction was performed under identical conditions, but differed in that ring closure was between a D and an L residue. Compound **6**, obtained in this way, was converted to 4-*epi*-A83586C (**8**)[12] by the protocol described in Scheme 3.

Detailed structural studies have been carried out on **8** in $CDCl_3$ at 24 °C by NMR. Proton assignments were made using 2D-COSY, TOCSY and NOESY data at 400 and 500 MHz. Conformational information was extracted from direct measurement of vicinal coupling constants and NOEs present in the 2D-NOESY and 1D-DPFGSENOE[13] spectra; mixing times were in the range of 100-300 msec. Structures were generated using restrained molecular dynamics and simulated annealing

(Molecular Simulations Inc., INSIGHT/DISCOVER). A representative, partially-refined, structure which illustrates several interesting features is shown in Figure 2.

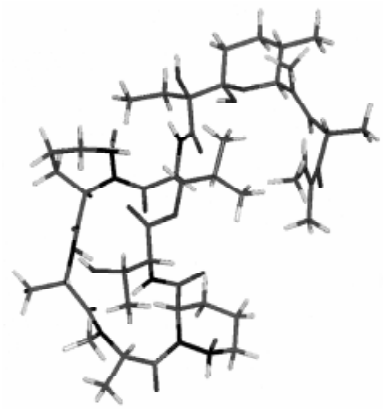


Figure 2

The presence of a strong NOE between H(4) and H(9) indicates that the C(8)-carbonyl group is *cis* to the piperazic acid A-ring NH in **8**. This contrasts markedly with A83586C itself, where the C(8)-carbonyl is *trans* to this NH. The remaining amide bonds in **8** all have the same configuration as A83586C. NOEs from the N(OH) to H(4) and H(13) point to the *N*-hydroxyl being endocyclic in **8**. The orientation of the ester linkage is not well-defined in our structure, due to the lack of proton restraints. Analysis of vicinal coupling constant and NOE data shows that both piperazine α-protons, H(4) and H(13), are essentially equatorial in **8**. The D-threonine and L-hydroxyleucine side-chains are restricted to the rotamers shown by the small couplings between H(19) and H(24) ($J_{H19,24}$ = 1.5 Hz) and between H(2) and H(20) ($J_{H2,20}$ = 1.6 Hz), with rotational-averaging being minimal. In the pyran ring, the axial position of H(34) is apparent from its large coupling with H(33). The weak NOE between H(34) and the C(30)-hydroxyl also confirms that the latter is axial. The unsaturated side chain in **8** is restrained by weak NOEs between H(36) and the hydroxyleucine methyls, as well as strong NOEs between H(37) and H(40), and H(36) with H(34). Work is continuing on refining this structure further, and the results of these efforts will be the subject of a future full publication.

To conclude, our total synthesis of 4-*epi*-A83586C has provided a further demonstration of the generality and power of the biomimetic BtO ester coupling strategy for constructing molecules[14] of the A83586C genre. Our work has also shown that macrolactamisation of the A83586C hexapeptide precursor **2** is accompanied by an epimerisation in the L-piperazic acid moiety when it is mediated by excess BOP and DMAP at high dilution. Finally, our 2D NMR and molecular modelling studies on **8** have allowed a preliminary, partially-refined, solution structure to be proposed, in which the C(8)-carbonyl group is oriented *cis* to the NH of the piperazine A-ring. Further studies in each of these areas will be reported shortly.

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12. All new compounds gave satisfactory 400 MHz ^{1}H and 100 MHz ^{13}C NMR and IR spectral data as well as HRMS data. (**8**) is an an amorphous solid: $[\alpha]_D$ +43.6 ^{o} (*c* 0.5 CH_2Cl_2); 500 MHz ^{1}H NMR (24 ^{o}C, $CDCl_3$, chemical shifts reported relative to residual $CHCl_3$ peak at δ 7.24) δ 9.16 (d, *J* = 8.1 Hz, ThrNH), 6.56 (br, ThrOH), 1.75 (br, PipNH-Ring A), 8.89 (s, NOH), 6.13 (d, *J* = 12.4 Hz, PipNH-Ring B), 9.30 (d, *J* = 10.3 Hz, HLeuNH), 4.47 (dd, *J* = 1.6, 8.1 Hz, H2), 4.40 (dd, *J* = <2.0, 5.7 Hz, H4), 2.02 (m, H5a), 1.78 (m, H5b), 1.89 (m, H6a), 1.70 (m, H6b), 3.11 (m, H7a), 2.75 (m, H7b), 5.47 (q, *J* = 6.6 Hz, H9), 5.39 (q, *J* = 6.6 Hz, H11), 5.70 (dd, *J* = < 4.0, < 4.0 Hz, H13), 1.88 (m, H14a), 1.79 (m, H14b), 1.55 (m, H15a), 1.29 (m, H15b), 3.09 (m, H16a), 2.75 (m, H16b), 4.80 (dd, *J* = 10.3, 10.3 Hz, H18), 4.92 (dd, *J* = 1.5, 10.4 Hz, H19), 4.69 (m, H20), 1.30 (d, *J* = 6.7 Hz, H21-Me), 1.28 (d, *J* = 6.8 Hz, H22-Me), 1.32 (d, *J* = 6.6 Hz, H23-Me), 1.79 (m, H24), 0.58 (d, *J* = 6.9 Hz, H25-Me), 0.76 (d, *J* = 6.7 Hz, H26-Me), 2.97 (s, H27-NMe), 2.87 (s, H29-OH), 6.31 (s, H30-OH), 1.69 (m, H31a), 1.59 (m, H32a), 1.41 (m, H33), 3.97 (d, *J* = 10.2 Hz, H34), 5.69 (d, *J* = 9.0 Hz, H36), 4.08 (dq, *J* = 7.1, 8.9 Hz, H37), 6.71 (q, *J* = 6.8 Hz, H40), 1.80 (d, *J* = 6.8 Hz, H41-Me), 1.99 (m, H42a), 1.58 (m, H42b), 0.86 (t, *J* = 7.4 Hz, H43-Me), 0.68 (d, *J* = 6.5 Hz, H44-Me), 1.55 (s, H45-Me), 1.09 (d, *J* = 7.0 Hz, H46-Me), 1.71 (s, H47-Me), H31b and H32b resonate between 1.50 and 1.70 ppm but are not assigned due to resonance overlap; 100 MHz ^{13}C NMR ($CDCl_3$) δ 203.1, 174.9, 173.4, 172.9, 169.0, 168.0, 167.6, 165.5, 137.3, 136.7, 132.8, 129.0, 99.5, 82.6, 80.2, 79.0, 66.8, 58.4, 56.4, 56.3, 51.1, 50.3, 48.0, 47.3, 45.3, 38.2, 32.6, 30.2, 28.5, 27.2, 27.13, 27.08, 25.8, 24.0, 21.2, 20.4, 20.0, 19.7, 19.5, 17.7, 15.2, 14.9, 14.1, 14.0, 11.6, 11.4, 8.5; HRMS Calcd for $C_{47}H_{76}O_{14}N_8Na$ $[M+Na]^{+\cdot}$ *m/z* 999.5379, Found: *m/z* 999.5350.
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